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Antioxidant capacity of fresh-cut vegetables exposed to ionizing radiation†‡

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Abstract: The effect of ionizing radiation on antioxidant capacity, phenolic content and tissue browning of three vegetables was studied. Midrib and non-midrib leaf tissues of Romaine and Iceberg lettuce and endive were irradiated with gamma-rays at 0, 0.5, 1 and 2 kGy, and then stored at 7–8 °C for 8 days. Antioxidant capacity and phenolic content of tissues as well as tissue browning were analyzed at 1, 4 and 8 days of storage. In general, irradiation increased the phenolic content and antioxidant capacity of both tissue types of all vegetables at day 4 and day 8. The rates of the increase were higher in midrib tissues than in non-midribs, and increased with storage time. Irradiation, however, increased tissue browning of midrib tissues of Romaine and Iceberg lettuce. Our results suggest that irradiation increased nutritional quality of leafy vegetables, but some adverse visual quality changes were encountered.
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Keywords: antioxidant capacity; phenolics; ionizing radiation; lettuce; endive

INTRODUCTION

Ionizing radiation inactivates foodborne pathogens, improves hygienic quality and extends the shelf-life of many fresh fruits and vegetables.[1] Fruits and vegetables are rich in antioxidants which exhibit a wide range of biological, pharmacological and chemoprotective properties that prevent cancer and reduce mortality due to cancer and heart diseases.[2] The beneficial effects of fresh fruits and vegetables are partially due to high amounts of antioxidants including phenolic compounds. Irradiation increases phenolic content in a number of plant tissues. Riov *et al*[3] found that gamma irradiation (2 kGy) of citrus fruit resulted in a rapid accumulation of phenolic compounds in peel tissues. The authors suggested that the increase in phenolic compounds was due to the increased phenylalanine ammonia-lyase (PAL) activity. Later Riov[4] showed phenolic compounds, which accumulated in flavedo cells following irradiation, caused cell death and consequent peel necrosis (pitting). Similarly, Oufedjikh *et al*[5] found increased levels of phenolic compounds, such as hesperidin and *p*-coumaric acid, in irradiated (0.3 kGy) citrus peel after 14 days of post-irradiation storage at 3–4 °C. Benoit *et al*[6] found that ionizing radiation transiently increased phenolic content in fresh mushrooms during a 9-day post-irradiation storage period at 4 °C; the phenolic content of irradiated samples increased rapidly initially (1–2 days), and then decreased to a level similar to the control at day 3 and thereafter. Mondy and Gosselin[7] found that 0.1 and 1 kGy gamma radiation increased phenolic content and discoloration of potatoes. Breitfeliner *et al*,[8] using doses up to 10 kGy, found that irradiation mainly increased the content of 4-hydroxybenzoic acid among the phenolics in fresh strawberry fruit.

Despite a number of studies showing the increase of phenolic content in several fresh fruits and vegetables, the effect of irradiation on total antioxidant capacity of various fruits and vegetables is not well documented. Most of the earlier studies focused on the relationship between enzyme activity and accumulation of phenolic compounds, and also on that between phenolic compounds and radiation-induced injuries. Studies on the correlation between irradiation and changes in phenolic and total antioxidant capacity in fresh-cut leafy vegetables are lacking. It is also unclear whether there is a different response in the changes in phenolic content and in antioxidant capacity of leaf tissues. If irradiation increases antioxidant capacity in fresh-cut vegetables, it will lead to more nutritive and, therefore, healthier products for consumers. Our earlier studies suggested that irradiation increased antioxidant capacity in alfalfa sprouts[9] and fresh-cut Iceberg lettuce.[10] The objectives of this study were

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to investigate the effect of low dose irradiation on antioxidant capacity, phenolic content and visual quality of three types of common fresh-cut vegetables, and to study the response of different tissue types to irradiation.

MATERIALS AND METHODS

Sample preparation

Romaine and Iceberg lettuce (*Lactuca sativa* L) and endive (*Cichorium endiva* L) were purchased from a local supermarket. Wrapper leaves were discarded, and only undamaged mature leaves were used. There were two types of leaf tissues selected: midrib and non-midribs (ie leaf blade without midrib tissue). Midrib tissues were excised starting about 2–3 cm from the base of each leaf. The segments were about 2 cm wide, and 2 cm in length for Iceberg and Romaine lettuce. For endive, the segments were about 1 cm wide and 2 cm long. The non-midrib tissues were also cut into pieces (2 cm square). The midrib and non-midrib pieces were dipped in cold (7 °C) deionized water for 1 min and centrifuged using a hand-held salad spinner (Wilton Industries Inc, Woodridge, IL) to remove surface water. The pieces were then placed in perforated film bags (E-300, Cryovac, Deerfield, IL,) and irradiated at different doses (0, 0.5, 1 and 2 kGy) using gamma rays at 7 °C. Each bag replicate had about 25 g of samples. After irradiation, all samples were stored at 7–8 °C for 8 days. Antioxidant and phenolics were measured at day 1, 4 and 8 of storage. Visual quality evaluation was also performed.

Irradiation and dosimetry

The samples were irradiated using a self-contained cesium-137 gamma-radiation source (Lockheed Georgia Company, Marietta, GA) with a dose rate of 0.095 kGy min^{-1}. Detailed descriptions on irradiation and dosimetry have been reported earlier.[11] During irradiation, temperature (7 ± 2 °C) in the radiation chamber was controlled by flushing the gas phase of liquid nitrogen into the upper portion of the chamber. To eliminate possible effects of nitrogen flushing during irradiation, bags of all treatments and controls were placed in the chamber with nitrogen flushing for the same total period (approximately 31 min), but varied in exposure time to radiation to achieve the desired doses. Measured doses were within 7% of targeted doses.

Antioxidant capacity

Samples (10 g) were homogenized with 20 ml of 70% methanol using a homogenizer (Virtishear, Virtis, Gardiner, NY) at a speed setting of 70 for 1 min. The homogenate was filtered through four-layers of cheesecloth and then centrifuged at approximately 12 000 *g* for 10 min at 5 °C in a Sorvall RC2-B refrigerated centrifuge (Kendro Laboratory Products, Newtown, CT). Extracts were either analyzed for antioxidant capacity or phenolic content either within 4 h at 5 °C or after 2 weeks storage at −76 °C. Antioxidant capacity in the supernatants was determined using the ferric reducing antioxidant power (FRAP) assay.[12] Briefly, 100-µl samples were mixed with 3 ml of FRAP reagent. The FRAP reagent was prepared daily by combining 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,5,-tripyridyl-*S*-triazine in 40 mM HCl, and 20 mM $FeCl_3$ in the ratio of 10:1:1 (v:v:v). The mixture was incubated at 23 °C for 30 min, then absorbance at 593 nm was measured with a spectrophotometer (Shimadzu UV-1601 spectrophotometer, Shimadzu Scientific Instruments, Columbia, MD). FRAP values were calculated from $FeSO_4$ standard curves, and expressed as µmol g^{-1} FRAP values.

Phenolics analysis

Total phenolic content was measured using the Folin–Ciocalteu colorimetric method.[13] After proper dilution, the diluted extract (0.1 ml) used for the FRAP assay was mixed with 0.2 ml of Folin–Ciocalteu reagent (Sigma Chemical Co, St Louis, MO), and incubated for 1 min at 23 °C. Then 3 ml of 5% Na_2CO_3 was added. Absorbances at 760 nm were recorded for the mixtures after 2 h incubation at 23 °C. Phenolic content was expressed as µmol g^{-1} gallic acid (GA) equivalent after subtracting the contribution from ascorbic acid. Because ascorbic acid (a non-phenolic compound) reacts readily with Folin–Ciocalteu reagents and contributes to phenolic content, ascorbic acid in the extract was determined using an HPLC method.[10] After converting to GA equivalent, contribution of AA was subtracted from phenolic content. Under our test conditions, ascorbic acid at 100 µM gave GA equivalent of 56 µM.

Visual quality

Tissue browning was estimated based on appearance using a scale of 1–9, where 1 = no browning and 9 = severe browning. For each replicate sample, 10 pieces of samples were randomly evaluated by three judges who were familiar with the scoring system.

Experimental design and statistical analysis

The experiments were of completely randomized design. There were four replicates for each treatment/experiment. All experiments were performed twice on two different days. There were eight replicates for each treatment at any storage time. Although variation existed between experiments and the values are generally higher in the second experiment, identical trends were detected, therefore antioxidants capacity and phenolics were reported as percentage of non-irradiated controls. Data were subjected to statistical analysis using SAS ver 6.12 (SAS Institute, Cary, NC., USA). Effect of dose was assessed using both the least significant difference (LSD) analysis of general linear model and regression analysis.

RESULTS AND DISCUSSION

Table 1 shows changes in antioxidant capacity and phenolic content of non-irradiated fresh-cut vegetables during storage. Antioxidant capacity of non-irradiated midrib tissues of all vegetables increased during storage while no increase in antioxidant capacity was observed in non-midrib tissues (Table 1). Antioxidant capacity of non-irradiated Romaine non-midribs slightly decreased during storage. Non-midrib tissues had much higher antioxidant capacity than midrib tissues for Romaine lettuce and endive. Phenolic content of midrib tissues of all non-irradiated vegetables increased during storage. In non-midrib tissues, phenolic content did not significantly ($p > 0.05$) change in Romaine lettuce, increased in Iceberg lettuce and decreased in endive during storage. Endive had much higher antioxidant capacity and phenolic content than Romaine and Iceberg lettuce while Romaine lettuce had higher antioxidant capacity and phenolic content than Iceberg lettuce. The increase in phenolic content and antioxidant capacity in midrib tissue is presumably a wounding response. It is known that wounding activates PAL and increases synthesis of phenolics.[14] Our results showed midrib tissues are much more responsive to wounding than non-midribs in terms of increases in antioxidant capacity and phenolic content.

Compared with non-irradiated controls, irradiation at all doses had no significant ($p > 0.05$) effect on antioxidant capacity of any tissue of the vegetables 1 day after irradiation (Table 2). On day 4, irradiation generally increased antioxidant capacity of both midrib and non-midrib tissues of all three leafy vegetables. However, linear increases over radiation dose were observed only in the midrib tissues of Iceberg lettuce and endive. The increases were 12.9, 22.1 and 13.5% per kGy radiation in Romaine, Iceberg lettuce and endive, respectively. After 8 days of storage, irradiated midrib tissues of all vegetables had higher antioxidant capacity than the corresponding non-irradiated tissues. In non-midrib tissues, irradiation only promoted antioxidant capacity of Iceberg lettuce. No effect by irradiation was observed in non-midrib tissues of Romaine lettuce or endive. Relative to the non-irradiated controls, the increases in antioxidant capacity of midrib tissues of Romaine and Iceberg lettuce and endive were 25.8, 38.2 and 14.5% per kGy of radiation, respectively.

No significant effect on phenolic content of any tissue of the vegetables by irradiation was observed 1 day after irradiation except that a linear increase over radiation dose was observed for Iceberg midribs (Table 3). Four days after irradiation, phenolic content of midrib tissues of all vegetables increased linearly with radiation dose. The increases were 16.9, 17.3 and 18.7% per kGy of radiation in Romaine, Iceberg lettuce and endive, respectively. Phenolic content of non-midrib tissues was not significantly ($p > 0.05$) affected by irradiation. After 8 days of storage, phenolic content of midrib tissues of all vegetables increased as radiation dose increased. Compared with non-irradiated samples, the increases were 20.3, 32.6 and 14.5% per kGy of radiation in midrib tissues of Romaine and Iceberg lettuce and endive, respectively. Linear increases in phenolic content were also observed in non-midrib tissues of Iceberg lettuce. In endive non-midrib tissues, only 1 kGy radiation significantly induced phenolic content.

The magnitude of irradiation-induced antioxidant capacity and phenolic content in midrib tissue increased with storage time, suggesting phenolics were synthesized during storage. For example, at day 1, the effect of irradiation on antioxidant capacity and phenolic content was generally not significant. At day 4, linear increases over radiation dose were observed in midribs of most vegetables. At day 8, phenolic content of all midrib tissues increased with radiation dose. The irradiation-induced increases in antioxidant capacity of Iceberg midribs were higher at day 8 (38.2% per kGy) than at day 4 (22.1% per kGy). Similarly the increases in phenolic content of Iceberg midribs were

Table 1. Changes in antioxidant capacity and phenolic content of non-irradiated Romaine and Iceberg lettuce and endive leaf tissues[a,b]

	Romaine		Iceberg		Endive	
Days	Midrib	Non-midrib	Midrib	Non-midrib	Midrib	Non-midrib
			FRAP (μmol g^{-1} FW)			
1	0.72 ± 0.05 a	8.83 ± 0.15 b	0.44 ± 0.02 a	0.68 ± 0.10 a	1.14 ± 0.08 a	18.75 ± 2.12 a
4	0.94 ± 0.07 b	7.06 ± 2.45 ab	0.72 ± 0.05 b	0.62 ± 0.11 a	1.94 ± 0.12 b	20.50 ± 2.17 a
8	1.10 ± 0.11 c	6.77 ± 1.62 a	0.85 ± 0.12 c	0.66 ± 0.06 a	2.29 ± 0.29 c	18.68 ± 1.36 a
Linearity[c]	***	*	***	NS	***	NS
			Phenolic content (μmol GA g^{-1} FW)			
1	0.40 ± 0.04 a	0.75 ± 0.18 a	0.05 ± 0.01 a	0.09 ± 0.01 a	0.62 ± 0.12 a	2.57 ± 0.63 a
4	0.98 ± 0.17 b	0.72 ± 0.25 a	0.11 ± 0.01 b	0.09 ± 0.01 a	1.25 ± 0.18 b	2.03 ± 0.22 b
8	1.11 ± 0.16 c	0.81 ± 0.13 a	0.18 ± 0.02 c	0.15 ± 0.02 b	2.00 ± 0.24 c	1.84 ± 0.04 b
Linearity[c]	***	NS	***	**	***	*

[a] Antioxidant capacity and phenolic content were measured using the FRAP assay and Folin–Ciocalteu assay, respectively, at 1, 4 and 8 days of storage at 7–8 °C. The numbers are means followed by standard deviations ($n = 4$).

[b] Means in column with same letters are not significantly different (LSD, $p < 0.05$). Comparison was made within same vegetables and tissues.

[c] NS, *,** and *** indicate no significance or significance of linear regression at $p < 0.05$, 0.01 and 0.001 levels, respectively.

Table 2. Effect of ionizing radiation on antioxidant capacity of Romaine and Iceberg lettuce and endive leaf tissues[a,b]

	Romaine		Iceberg		Endive	
Dose (kGy)	Midrib	Non-midrib	Midrib	Non-midrib	Midrib	Non-midrib
			1 day of storage			
0	100 ± 6 a	100 ± 7 a	100 ± 3 a	100 ± 12 a	100 ± 7 ab	100 ± 8 a
0.5	99 ± 5 a	99 ± 26 a	104 ± 4 a	99 ± 10 a	91 ± 12 a	113 ± 11 a
1.0	97 ± 6 a	108 ± 29 a	105 ± 6 a	102 ± 22 a	108 ± 18 b	111 ± 20 a
2.0	103 ± 7 a	105 ± 30 a	104 ± 7 a	104 ± 14 a	96 ± 13 ab	105 ± 22 a
Linearity[c]	NS	NS	NS	NS	NS	NS
			4 days of storage			
0	100 ± 5 a	100 ± 33 a	100 ± 6 a	100 ± 9 a	100 ± 6 a	100 ± 9 a
0.5	123 ± 23 ab	161 ± 66 b	117 ± 29 ab	110 ± 22 ab	107 ± 8 a	121 ± 18 bc
1.0	141 ± 61 b	184 ± 53 b	123 ± 33 ab	125 ± 13 b	116 ± 5 b	114 ± 12 ab
2.0	131 ± 11 ab	154 ± 24 b	147 ± 41 b	101 ± 9 a	127 ± 8 c	119 ± 13 bc
Linearity	NS	NS	***	NS	***	NS
			8 days of storage			
0	100 ± 9 a	100 ± 22 a	100 ± 11 a	100 ± 5 a	100 ± 12 a	100 ± 7 a
0.5	114 ± 12 b	119 ± 18 a	138 ± 17 b	113 ± 20 a	119 ± 10 b	107 ± 9 a
1.0	125 ± 9 c	136 ± 58 a	177 ± 52 c	120 ± 26 ab	115 ± 9 b	105 ± 13 a
2.0	152 ± 18 d	122 ± 21 a	188 ± 29 c	135 ± 21 b	134 ± 17 c	109 ± 12 a
Linearity	***	NS	***	***	***	NS

[a] Midrib and non-midrib leaf tissues were irradiated with 0, 0.5, 1 and 2 kGy gamma-rays, and stored at 7–8 °C Antioxidant capacity was measured using the FRAP assay at 1, 4 and 8 days after irradiation, and expressed as percentage of the non-irradiated controls. The numbers are means followed by standard deviations ($n = 8$).

[b] Means in column with same letters are not significantly different (LSD, $p < 0.05$). Comparison was made within same storage days.

[c] NS and *** indicate no significance or significance of linear regression at $p < 0.05$, 0.01 and 0.001 levels, respectively.

also higher at day 8 (32.6% per kGy) than day 4 (17.3% per kGy).

On average, tissue browning increased during storage in midrib tissues of all samples (Table 4). More severe browning was observed in midrib tissue than non-midrib tissues. One day after irradiation, no significant ($p > 0.05$) browning in any tissue of the vegetables was observed (data not shown). After 4 days of storage, irradiation increased tissue browning of non-midrib tissues of all three leafy vegetables except Romaine lettuce where the effect was not significant. After 8 days of storage, irradiation-induced tissue browning was observed in both midrib and non-midrib tissues of Iceberg lettuce and midribs of Romaine. Moderate to severe browning was observed in irradiated Iceberg midrib tissue. Tissue browning of Romaine non-midrib tissues decreased with increasing radiation dose. Irradiation did not significantly ($p > 0.05$) increase tissue browning of endive midrib and non-midrib tissue.

The non-midrib tissues had a higher phenolic content and antioxidant capacity than midrib tissues in Romaine lettuce and endive (Table 1). Although irradiation generally increased antioxidant capacity values and phenolic content of both midrib and non-midrib tissues, the increases (in terms of percentage of non-irradiated) in antioxidant capacity and phenolic content were more pronounced in midrib tissues than in non-midrib tissues. Furthermore, tissue browning was more severe in midrib than in non-midrib tissues. Ionizing radiation exerts its effects mainly through free radicals generated from radiolysis of water.[15] In response to abiotic or biotic stresses, such as radiation, plant tissues are able to adapt to the adverse conditions via increasing endogenous antioxidants, including phenolics. It seems that tissues with higher antioxidant capacity showed less response to irradiation while tissues with lower antioxidant capacity responded strongly. Iceberg lettuce had lower antioxidant capacity than endive and Romaine lettuce. Consequently, the percentage increase in antioxidant capacity was generally higher in Iceberg lettuce than endive and Romaine.

Although irradiation, in general, increased antioxidant capacity of all tested vegetables, especially for midrib tissues, the increased phenolics may also contribute to the browning of tissues as evidenced on Iceberg lettuce. Measures must be taken to reduce tissue browning for Iceberg lettuce. Modified atmosphere packaging, heat shock or anti-browning chemicals may be used in combination with irradiation to reduce the adverse affects of irradiation.

Phenolic compounds are generally synthesized by the shikimate pathway in which PAL is the key enzyme. Similar to mechanical wounding,[16] irradiation can increase PAL activity of plant tissue,[17] resulting in the accumulation of phenolic compounds. Major antioxidants in fresh vegetable are phenolic acids and flavonoids.[18] Phenolics, such as tannic acid and gallic acid, have high antioxidant activity.[19] Irradiation increased both phenolics content and antioxidant capacity, suggesting the increased phenolics synthesis contributed to the total antioxidant capacity.

It is also possible that the increased antioxidant capacity is related to tissue browning. The enzymatic browning is defined as an enzymatic oxidation of

Table 3. Effect of ionizing radiation on phenolic content of Romaine and Iceberg lettuce and endive leaf tissues[a,b]

	Romaine		Iceberg		Endive	
Dose (kGy)	Midrib	Non-midrib	Midrib	Non-midrib	Midrib	Non-midrib
			1 day of storage			
0	100 ± 6 a	100 ± 15 a	100 ± 7 a	100 ± 12 a	100 ± 41 a	100 ± 19 a
0.5	112 ± 8 a	101 ± 35 a	107 ± 6 a	103 ± 23 a	93 ± 27 a	92 ± 27 b
1.0	102 ± 27 a	112 ± 38 a	116 ± 13 a	102 ± 20 a	116 ± 23 a	114 ± 36 ab
2.0	96 ± 35 a	111 ± 37 a	141 ± 46 b	112 ± 18 a	119 ± 35 a	105 ± 30 ab
Linearity[c]	NS[c]	NS	*	NS	NS	NS
			4 days of storage			
0	100 ± 9 a	100 ± 35 a	100 ± 7 a	100 ± 13 a	100 ± 7 a	100 ± 9 a
0.5	113 ± 18 ab	100 ± 24 a	120 ± 10 b	121 ± 26 b	117 ± 13 b	113 ± 7 b
1.0	110 ± 12 ab	128 ± 36 a	132 ± 7 c	125 ± 9 b	127 ± 17 bc	104 ± 13 ab
2.0	136 ± 16 b	122 ± 15 a	139±c b	111 ± 7 ab	139 ± 18 c	105 ± 10 ab
Linearity[c]	**	NS	***	NS	***	NS
			8 days of storage			
0	100 ± 12 a	100 ± 14 a	100 ± 9 a	100 ± 12 a	100 ± 12 a	100 ± 12 a
0.5	109 ± 8 a	106 ± 8 a	106 ± 5 a	103 ± 16 a	115 ± 7 b	131 ± 32 ab
1.0	123 ± 14 b	108 ± 34 a	140 ± 13 b	106 ± 19 ab	114 ± 17 b	133 ± 39 b
2.0	140 ± 27 c	103 ± 18 a	160 ± 22 c	121 ± 16 b	125 ± 14 c	128 ± 31 ab
Linearity	***	NS	***	*	**	NS

[a] Midrib and non-midrib leaf tissues were irradiated with 0, 0.5, 1 and 2 kGy gamma-rays, and stored at 7–8 °C. Phenolic content was measured using the Folin–Ciocalteu assay at 1, 4 and 8 days after irradiation, and expressed as percentage of the non-irradiated controls. The numbers are means followed by standard deviations ($n = 8$).

[b] Means in column with same letters are not significantly different (LSD, $p < 0.05$). Comparison was made within same storage days.

[c] NS, *,** and *** indicate no significance or significance of linear regression at $p < 0.05$, 0.01 and 0.001 levels, respectively.

Table 4. Effect of ionizing radiation on tissue browning of Romaine, Iceberg lettuce and endive leave tissues[a,b]

	Romaine		Iceberg		Endive	
Dose (kGy)	Midrib	Non-midrib	Midrib	Non-midrib	Midrib	Non-midrib
			4 day of storage			
0	1.3 ± 0.5 a	1.1 ± 0.4 a	2.9 ± 1.6 a	2.0 ± 0.1 a	2.0 ± 0.6 a	1.0 ± 0.0 a
0.5	1.6 ± 0.5 a	1.0 ± 0.0 a	2.9 ± 0.9 a	3.1 ± 0.4 b	1.9 ± 0.7 a	1.0 ± 0.0 a
1.0	1.5 ± 0.5 a	1.3 ± 0.5 a	4.5 ± 1.0 b	3.0 ± 0.2 b	2.2 ± 0.8 a	1.0 ± 0.0 a
2.0	2.3 ± 1.1 b	1.0 ± 0.1 a	4.8 ± 1.1 b	3.0 ± 0.1 b	2.9 ± 0.9 b	1.8 ± 0.5 b
Linearity[c]	**[c]	NS	***	***	**	**
			8 days of storage			
0	2.1 ± 0.6 a	2.9 ± 0.4 b	2.8 ± 1.2 a	2.0 ± 0.1 a	3.4 ± 0.5 a	2.9 ± 0.8 a
0.5	2.3 ± 0.6 ab	2.8 ± 0.5 b	4.5 ± 0.8 b	2.8 ± 0.3 b	3.2 ± 0.5 a	3.3 ± 0.5 a
1.0	2.4 ± 0.8 ab	2.0 ± 0.1 a	5.9 ± 0.5 c	2.9 ± 0.2 b	3.2 ± 0.7 a	3.1 ± 0.6 a
2.0	2.8 ± 1.1 b	2.0 ± 0.1 a	7.3 ± 0.5 d	3.4 ± 0.2 c	3.4 ± 0.8 a	2.8 ± 0.9 a
Linearity[c]	*	***	***	***	NS	NS

[a] Midrib or non-midrib leaf tissues were irradiated with 0, 0.5, 1 and 2 kGy gamma-rays, and stored at 7–8 °C. Browning was evaluated at 4 and 8 days after irradiation. The numbers are means followed by standard deviations ($n = 8$). Tissue browning was rated on a 1–9 scale, where 1 = no browning and 9 = severe browning

[b] Means in column with same letters are not significantly different (LSD, $p < 0.05$). Comparison was made within same storage days.

[c] NS, *,** and *** indicate no significance or significance of linear regression at $p < 0.05$, 0.01 and 0.001 levels, respectively.

phenols into slightly colored quinones. Polyphenol oxidase (PPO) catalyzed hydroxylation of monophenols to *o*-diphenol and dehydrogenation of *o*-diphenol to *o*-quinone. The quinones then polymerize, leading to the formation of pigments. It has been shown that the early stage of polymerization, in which dimers and trimers are formed, results in increased antioxidant capacity.[20,21] Irradiation, in general, increased PPO and peroxidase capacity in fresh fruits and vegetables.[17] The increased PPO activity may result in accelerated rate of *o*-quinone formation and synthesis of dimers and trimers. The exact mechanism(s) for the irradiation-induced antioxidant capacity in the fresh-cut vegetables needs further study.

It is well known that irradiation inactivates foodborne pathogens in various vegetables, resulting in improved microbial food safety of fresh-cut vegetables.[22] Our results suggest irradiation also increased nutritive values by promoting production

of antioxidants. Currently, US authorities allow use of irradiation on fresh fruits and vegetables at a dose of 1 kGy.[23] At the current permitted maximum dose (1 kGy), the antioxidant capacity of the vegetables can be increased by 14%.

CONCLUSIONS

Our results suggest that irradiation increased phenolic content and antioxidant capacity of endive, Romaine and Iceberg lettuce. The increase was more profound in midrib tissue than non-midrib tissues. Increased consumption of diets rich in antioxidants and phenolics may contribute to reducing human diseases, and irradiation processing may produce a healthier vegetable product. However, the increased phenolics may also increase tissue browning for some vegetables. Thus a combination of anti-browning measures with ionizing radiation may be needed for some vegetables.

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